FINAL REPORT

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TITLE OF GRANT:Assessing the Importance of Calcite to Optical Backscattering in the Ocean

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GOALS: The long-term goals for my ONR project were to quantify the light scattering properties of suspended calcite particles in the sea, and to predict suspended calcite concentrations in space and time. Calcite is one of the most abundant minerals on earth, and much of it is biogenically formed by ubiquitous phytoplankton known as coccolithophores. These picoplankton produce micron-sized scales known as coccoliths which represent a major source of optical scattering in the sea. Understanding their variability will allow more complete optical closure in the sea.

OBJECTIVES: The objectives of my work during the first year were to: 1) measure the volume scattering function of solutions of detached coccoliths from various cultured coccolithophore species, 2) calculate optical backscattering for the individual detached coccoliths, 3) measure the calcium content of the same samples using graphite-furnace atomic absorption in order to calcite-specific backscattering coefficient and 4) collect calcite samples from ships of opportunity for sorting of natural calcite particles.

The objectives of the second year's experiments were to measure the calcite-specific backscattering coefficient for pure biogenic calcite coccoliths sorted from 1) pure cultures of various species of coccolithophores and 2) natural seawater samples containing a variety of coccoliths not available from cultures.

APPROACH: This work has combined flow cytometry, volume scattering measurements, and graphite furnace atomic absorption measurements, for quantifying scattering properties of calcareous algae. Specifically, I used the Bigelow Laboratory Epics V Flow Cytometer to sort detached coccoliths from cultures at the Bigelow Laboratory Culture Collection. Next, I measured the volume scattering functions of the pure sorts and calculated the particle-specific backscattering. Each of these samples were then filtered for subsequent measurements of calcite content using atomic absorption. The field component of this research involved the first sea-trials of our 18 channel light-scattering photometer which we used to estimate calcite specific backscattering in the Straits of Florida, a region known for its vast carbonate banks and high amounts of suspended calcite. During this work, I found that the backscattering coefficient for a large variety of biogenic calcite-covered cells changed by almost two orders of magnitude when calculated as the backscattering *per unit cell* (m^2/cell). I hypothesized that if backscattering coefficients of calcite covered cells were calculated *per unit mass calcite*, then the backscattering coefficient would be much less variable, regardless of species.

The approach then used in the second year's experiments was to measure the calcite-specific backscattering coefficient for pure biogenic calcite particles. Recall that the limitation of the first year experiments was that the flow cytometer could only sort *plated coccolithophore cells*, where we gated on chlorophyll a fluorescence and side scatter. Thus, the cell sorts contained both particulate organic and inorganic matter, not pure calcite. The instrument still could not sort non-fluorescing, scattering particles, free from organic matter. This was particularly obvious in our measurements of the calcifying dinoflagellate, *Thoracosphaera* sp, in which the presence of cellular protoplasm in the calcite thecae

significantly altered the scattering coefficient of the cell. Therefore, the definitive experiment was done in the second year in which light scattering of pure coccolith suspensions was examined. This required major enhancements of our analytical abilities, both in the flow cytometry and the calcite detection.

TASKS COMPLETED
(Oct'94-Oct '95):

I finished the initial phase of the scattering analyses on suspensions of detached coccoliths sorted using the EPICS flow cytometer. Linearity between the scattering and calcite concentration was achieved on each species. I also successfully used a dual forward angle sensor for examining birefringence properties of each particle for better discrimination of coccoliths. Each sample was then filtered onto nuclepore filters for subsequent atomic absorption analyses.

A continuous culture experiment with Emiliania huxleyi was completed, that was designed to examine coccolith size and associated optical properties as a function of nutrient-limited growth rate.

I took our Dawn laser light scattering photometer to sea in January for its first field trials. The cruise in the Florida Straits (in very rough seas) was designed to cover a wide range of suspended calcite concentrations. Temperature, salinity, pH, fluorescence, and volume scattering were measured simultaneously by this device.

(Oct'95-Oct '96):

Lab Experiments

I ran a series of flow cytometer experiments at Bigelow Laboratory in late '95. The goal of the experiments was to sort individual coccoliths with the flow cytometer, to measure the volume scattering properties of the sorts, then to measure the mass of calcite within the sort. Sorting of individual coccoliths is not trivial with the flow cytometer due to their small size and lack of autofluorescence, and it required careful tuning of the instrument so that we could sort coccoliths based on the ratio of horizontally- and vertically-polarized forward light scattering. Moreover, this work required much more precise measurements of calcium concentration, heretofore never made on these calcifying algal species. This was particularly difficult when one considers that seawater (and flow cytometer sheath fluid) has Ca^{++} concentrations in excess of 10 mM. We ran our samples from this experiment on a a Perkin Elmer Model 5100PC graphite furnace atomic absorption spectrometer, with three orders of magnitude more sensitivity (50 pg Ca ml^{-1}) than flame atomic absorption. As an example of the experiments, we sorted 100,000 coccoliths of *Emiliania huxleyi* (2mm diameter) with the flow cytometer. This translated to ~25 ng C or 83 ng Ca, which, in a 5 mL final volume, gave a concentration of 16.6 ng Ca ml^{-1} (still sufficient to give a signal to noise ratio >300 on the graphite furnace atomic absorption spectrometer).

Species of calcifying algae (both coccolithophores and *Thoracosphaera heimii*, the calcifying dinoflagellate) were purchased from the Provasoli-Guillard Culture Collection (at Bigelow Laboratory), and grown in K media. Cultures were kept in the temperature-controlled rooms at Bigelow on a 14h:10h light:dark cycle and harvested in logarithmic growth phase for sorting with the EPICS V flow cytometer with multi-parameter data acquisition. Calcite particles then were sorted based on their birefringence under the laser light. Polarizing filters were placed, at right angles, on the two forward-angle scatter detectors of the flow cytometer (Olson et al. ,1989). Olson showed that the ratio of horizontally to vertically polarized forward light scatter was about 3.0 for calcite particles and 1.0 for all other particle types (we have found a ratio closer to 12 for coccoliths using the Bigelow Laboratory flow cytometer). This proved highly effective for discriminating and sorting calcite particles.

I performed the necessary set-up, cell counts and cell sorting for five calcifying algal species. Two species, *Syracosphaera* sp.and *Coccolithus pelagicus* grew in clumps which caused problems in sorting individual coccoliths. The other three species, *E. huxleyi* (clone 89E), *Cricosphaera* sp., and *Thoracosphaera* sp. were adequate for our experiments. I spent part of our flow cytometer time tuning the flow cytometer for sorting individual coccoliths and verifying that flow cytometer counts versus regular cell counts were in good agreement. This was absolutely critical to our final results. One aspect that allowed this work to proceed more rapidly than previous experiments, however, was that there was no need to sort plated cells since the goal was to define the backscattering coefficient per mg of calcite carbon. The laser light scattering photometer was calibrated using an isotropic scattering standard supplied with the instrument, and frequently checked for any instrument drift with ultra-clean distilled water.

Underway system for detection of calcite-dependent scatter

We also performed some ship-board work in which we continuously measured volume scattering of surface populations. Our next-generation underway scatter system uses a Wyatt Technologies laser-light scattering photometer equipped with a flow-through cell to measure volume scatter at 18 angles. Integration of this signal in the backward direction allows us to calculate backscatter in real time. This last year, we performed considerable programing and instrument interfacing in order to streamline the data flow. The system now monitors chlorophyll fluorescence, pH, temperature, salinity and 18-channel volume scatter. A Global Positioning System was interfaced, as well. In January '95, we took our flow-through light scattering detector to sea for its first field trials. Despite some very rough seas, the instrument ran without problem, and we recovered four detailed transects across the Florida Straits for volume scattering (sampling 400 times per second, and averaging over 3-5 minute time scales). These average volume scattering functions were used to calculate the backscattering coefficients on the same time scale. Given the velocity of the ship, this translated to horizontal resolution of about 900m . (This can be shortened to 300m resolution with no problem). While returning to Miami, we scaled 3 orders of magnitude in b_b, most of it resulting from calcite particles being resuspended and sloughing off the carbonate banks of the SE Florida shelf. Such high concentrations of suspended calcite are similar to the most dense coccolithophore blooms that we have ever visited; this raises interesting questions about the importance of suspended calcite to overall light scatter in Case II waters near carbonate banks.

In October and November, we took the underway flow-through system to the Arabian Sea for a 35d cruise during the intermonsoon period. The instrument ran virtually flawlessly, and we collected continuous data of total backscattering and calcite-dependent backscattering over the 3500 km cruise track.

RESULTS:
(Oct'94-Oct '95):

The results from the flow cytometer experiments showed particle-specific backscattering coefficients consistent with previous measurements on other species. One of the most important aspects from this last experiment was the successful configuration of the Epics V flow cytometer for measuring birefringence properties of individual coccoliths of the various species. This proved to be a valuable diagnostic for sorting calcite particles and will be invaluable for our sorts of field material next year. Linearity of the backscattering versus concentration plots demonstrated clearly that the flow cytometer was able to sort sufficient numbers of coccoliths for light-scattering measurements. Purity of the sorts was confirmed in cell counts of the same material. Note, previously, we sorted

plated coccolithophore cells which is technically much easier. Suspended calcite concentrations of these samples will be determined following our return from the Arabian Sea. Preserved field samples have been received this last year from ships of opportunity, representing a multitude of stations around the globe. Calcite in these samples will be sorted for subsequent optical measurements next year.

Results from our underway system used in the Straits of Florida showed that the instrument was able to measure backscattering over 3 orders of magnitude dynamic range between the Gulf Stream and the most turbid, carbonate laden waters of the Miami River. As expected, the relationship between chlorophyll and light scatter was fundamentally different in the Case II coastal waters than the offshore Case I waters , as calcite concentrations increased. Moreover, calcite sediments over Bahama Bank were generally very course, hence there was little resuspension as compared to the Florida shelf waters, where the particles were much finer and accounted for a much larger fraction of the total backscattering. The Arabian Sea results with this instrumentation from a cruise in October '95 will provide the largest examination yet on the patchiness scales of suspended calcite in the sea.

(Oct'95-Oct '96):
Laboratory Experiments

The results from this year's experiments showed that, indeed, the coccolith-specific backscattering coefficients were much more variable than the calcite-specific backscattering coefficients. All comparisons of b_b particulate versus the concentration of Ca demonstrated much less variance than the coccolith-specific values. The best-fit average calcite-specific b_b^* value for these data was 11.7 m^2 (mol C)$^{-1}$ (std dev=+/- 3.2m^2 (mol C)$^{-1}$). Equally noteworthy, was the fact that the calcite-specific b_b^* (based on pure sorts of coccoliths) was about an order of magnitude *higher* than the values based on plated cells (thus containing both organic and inorganic matter). Our previous results in which plated coccolithophores were sorted gave calcite-specific b_b^* values averaging about 0.6 m^2 mol C^{-1}(std dev=+/- 0.36 m^2 mol C^{-1}). The presence of absorbing organic matter obviously reduced the quantity of scattered light detected by the scatterometer. Moreover, when examining plated coccolithophores, the calcite coccoliths covered significant numbers of intracellular organelles. When the calcite was dissolved away from the cells, the decrease in backscattering was less than would be observed if pure suspensions of coccoliths (with no organic matter) were dissolved. Such an observation only served to emphasize the importance of preparing pure coccolith suspensions in order to determine their b_b^*.

The confirmation of low variance in the calcite-specific b_b^* for a wide range of particle sizes has significant ramifications for the remote sensing of suspended calcite. Simply put, the results suggest that for remote reflectance measurements of calcite, one need not know the species (particle type) responsible in order to calculate the suspended load of calcite within about 25% accuracy. If our results had shown more species-specific variance in b_b^*, then one would have had to know the type of particle in order to calculate the amount of suspended calcite. Moreover, our results showed that b_b^* values were less size dependent than predicted from anomalous diffraction calculations on calcite spheres.

We performed the last phase of this work this last September, in which we sorted natural calcite particles sampled from ships of opportunity from many locations in the world ocean. These samples were sorted using the polarizing filters placed at right angles over the two forward-angle scatter detectors of the flow cytometer. This provided a more representative estimate of the backscattering coefficient for naturally-occuring calcite particles. The microscope counts of flow-cytometer sorted samples showed that we had

good sorting discrimination using gating based on cross-polarized forward-angle scattered light. We have completed the atomic absorption samples and are processing the data as of this writing.

Underway System Results

Arabian Sea

Our underway measurements allowed us to demonstrate that consistently 10-30% of the total backscattering in the Arabian Sea was acid-labile (i.e. originated from calcite). The flow-through instrument also logged statistics of the backscattering events every 4 minutes of the trip. The data showed that various water masses could be characterized by well-defined scattering statistics (e.g. a striking relationship between the standard deviation of the scattering events, and the average backscattering value was found, suggesting a changing role of rare, large scatterers in certain water masses over the cruise track).

Gulf of Maine

Relatively speaking, the Arabian Sea is not a region known for meso-scale coccolithophore blooms. We have waited eagerly for an opportunity to take the flow-through instrument into the Gulf of Maine where the coccolithophore, *Emiliania huxleyi* forms large blooms. In March and June, 1996, we had our first Gulf of Maine cruises in which the flow-through instrument was used. As expected, calcite-dependent backscattering was low in March, but it was still measurable. In June, we ran 2000 miles of transect in the Gulf of Maine with our flow-through light scattering photometer and may have observed the early stages of coccolithophore bloom development in Wilkinson Basin. Acid-labile scatter dropped over Georges Bank, and increased in the Northeast Channel, similar to previous blooms that we have observed. The observations are consistent with the calcite being produced in the more stable Wilkinson Basin with subsequent advection around the NE flank of Georges Bank. We are preparing for another Gulf of Maine cruise at the beginning of November aboard the R/V Albatross. As before, we will be taking the flow-through system.

ACCOMPLISHMENTS:

The field trials of the Dawn Laser Light scattering photometer in January were in extremely rough seas, yet the instrument performed flawlessly, making continuous measurements of calcite-dependent backscattering, total backscattering, fluorescence, temperature, salinity, and pH. This sets the stage for the much more extensive application of the instrument in the Arabian Sea. I am also proud of this year's flow cytometry results in which we were able to discriminate between a wide variety of calcite particle types using birefringence. This, combined with the successful sorting of these calcite particles, will provide the best estimate yet of the mass-specific backscattering coefficient of calcite (b_b^*; units m^2 g $CaCO_3^{-1}$).

The most significant impact of our work (from the standpoint of understanding the effect of suspended calcite on in-water optics) was on the calculation of the mass-specific calcite backscattering coefficient using sorts of pure coccoliths instead of plated coccolithophore cells. The analytical difficulties of such measurements were enormous, from detecting and sorting individual 1-2mm coccoliths with the flow cytometer, measuring the volume scattering of each sort, to measuring nanogram levels of Ca. Isolating calcite from other organic matter was worth the effort, given that we showed "organic contamination" would reduce the b_b^* estimate by an order of magnitude. Moreover, our illustration of a relatively invariant b_b^* [11.7 m^2 (mol C)$^{-1}$; std dev=+/- 3.2m^2 (mol C)$^{-1}$], will be essential for an algorithm to remotely detect the concentration of suspended calcite in the sea.

Another aspect of our work will have impact on understanding the distributions of one algal group, coccolithophorids, in the sea. We had highly successful deployments of our underway system for measuring total and calcite-dependent backscattering in the Straits of Florida, Arabian Sea and Gulf of Maine cruises. Note, the sea state of the Gulf of Maine in March, 1996, aboard the R/V Argo Maine, was so bad that much of the other planned work was cancelled, yet the underway system operated flawlessly throughout the entire trip! During our recent June cruise, the instrument successfully collected continuous volume scattering data over 2000 miles in the Gulf of Maine. These underway data sets give us our first glimpse of the time and space variability of calcite-dependent backscatter in a variety of ocean types. The instrument is sufficiently sensitive to estimate volume scatter in any marine habitat. It has provided b_b and b_b' measurements over 3 orders of magnitude range from blue water into rivers; our approach could have impact on the more general problem of measuring small-scale optical variability in turbid, case II waters.

TRANSITIONS ACCOMPLISHED

We began this project knowing that our objective-- to sort individual coccoliths, measure their volume scattering and calcite concentration--was a very difficult task, indeed. Nevertheless, the experiments surpassed our expectations in that the relationships between coccolith concentration and backscattering had least-squares linear fits with r^2 values of 0.99. The most challenging aspect, by far, was to run natural seawater samples through the flow cytometer and sort calcite particles free from other living and non-living particulate matter. Our experiences from the previous flow cytometer experiments allowed us to precisely tune the instrument so that this task was actually much easier.

As for our flow-through system to measure calcite-dependent backscattering, no such data had ever been acquired continuously in the sea, so we had few preconceptions concerning the possible patterns we might see. In fact, one of the surprises was the amount of total backscattering which is caused by calcite...from 10-30% in the Arabian Sea. It must be emphasized that these were "non-bloom" waters where coccolithophores made up a small percentage of the total phytoplankton biomass. Our results showed preference of coccolithophores to moderately stable regimes, and we frequently saw them on the warm side of fronts in regions of low chlorophyll. The highest populations were associated with 23.5 sigma-T density surfaces that "outcropped" at the surface.

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Abstract

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